THE RELATIVE SIGNIFICANCE

of

CARBONIC ACID AND AMMONIA ELIMINATION

in the

ACID INTOXICATIONS OF PREGNANCY.

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Bibliographical thesis

presented for partial credit

toward the A.M. degree.

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by

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I N T R O D U C T I O N .

THE RELATIVE SIGNIFICANCE OF CARBONIC ACID AND AMMONIA ELIMINATION IN THE ACID INTOXICATIONS OF PREGNANCY.

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Of the various normal functions of the animal body the state of pregnancy as an incident of the reproductive function shows a curious anomaly. That the reproductive functions are to be regarded as fundamental and, hence, as wholly normal in character is too obvious to require discussion.

That, on the other hand, during the state of pregnancy, certain disturbances of normal function form characteristic incidents of the condition, is a general and well-recognized fact. The severity of these functional disturbances varies profoundly with the individual, so that while in many instances they are so superficial as to be wholly without significance, in an appreciable percentage they assume a gravity of character which may seriously endanger not only the health, but even the life of both mother and child. The general picture of these anomalies is the same, the differences being those of degree and not of kind.

Among these functional disturbances may be classed the various toxemias, several different types of which are recognized as potentially incidental to the pregnant state. Of these the so-called acid intoxications form a relatively

common and highly important group. It is necessary here, again, to differentiate between two types of acid intoxication. In the first of these, an imperfect oxidation of one or more of the normal energy-producing food materials may be regarded as the basis of the so-called acidosis. It is distinguished by a typical group of symptoms, among which may be mentioned the elimination of the so-called acetone bodies - B-hydroxybutyric acid, acetoacetic acid, and acetone - by the kidneys, a lessening of the titratable alkalinity of the blood, the hydrogen ion content remaining practically unchanged, a diminution of the carbon dioxide content of the alveolar air and a marked increase of the ammonia content of the urine. This group of symptoms is further found in certain other pathological and abnormal physiological states. For example, it is found that the feeding of mineral acids to herbivorous animals will produce a lessening of the titratable alkalinity of the blood, a diminution in the alveolar carbonic acid, and an increase in the urine of the fixed bases of the animal body, as sodium, potassium, calcium, and magnesium. The generally accepted explanation of this phenomenon, is that the acid on passing into the blood stream unites with the alkaline elements of the blood, and through the very delicate regulation of salt concentrations conditioned by functional activity in the kidney, there is a definite loss of these alkaline elements, the normal function of which is to fix the carbon dioxide thrown off during the katabolic activity of the cell, and thus permit

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it to be eliminated by the lungs. Similar experiments with the carnivora fail to exhibit a loss of the fixed alkalies, but do show a marked increase in the ammonia content of the urine. As the ash of the food of carnivora is acid in contradistinction to the alkaline ash of herbivorous nutrition, there is a partial protection normally established in the former organism, Further, the protein intake of the first far exceeds in quantity and utility that of the second, large quantities of ammonia being readily available through deaminization. Ammonia, then, may be regarded as the body's natural protection against increased acid formation. With man, a mixed feeder, we find the ammonia protection available, and thus in the relationship toward acid intoxications the eliminative features approach more nearly the picture of experimental acidosis with the carnivora.

Through disturbances in the organism we find the formation of varying amounts of the acetone bodies, and depending upon the origin, one or another symptom complex resulting from the same. For example, the lowering of the titratable alkalinity of the blood, with the appearance of the acetone bodies, the complementary lessening of the alveolar carbon dioxide, the appearance of the acetone bodies in the urine, and a marked increase in the urinary ammonia, forma condition which develops as a preliminary to diabetic coma. Further, we find

the comatose state in direct relationship with the amount of organic acid in the urine, coma appearing with increasing acid and disappearing with diminution of the acid content. The reduction of the alkalinity of the blood is an accompanying factor of the foregoing, and the fact frequently observed that the administration of alkalies tends toward improvement is in complete harmony with the facts already cited. On the other hand, in the disturbances of the organism produced by inanition, large quantities of B-hydroxybutyric acid will be eliminated by the urine without any evidence of coma. The source of this acid becomes a matter of primary interest. In diabetes the existing condition is one of a failure on the part of the organism to utilize available carbohydrates; in short, through one or another cause, a carbohydrate inanition exists. In the second instance, that of inanition, it is found that the glycogen reserve of the body is first drawn on for body maintenance and in a short time is materially exhausted. As before, the condition producing the acid intoxications rests upon a carbohydrate inanition. From this it seems safe to infer that the carbohydrates are literally anti-ketogenic in character, and that the acid formation cannot result from their imperfect oxidation. The other two possibilities, namely, the protein and fat of our nutrition, are potentially both sources of the acetone bodies. In regard to the former, we find the appearance of this acidosis associated with wasting diseases, in the course of

which large amounts of body protein are katabolized. On the other hand, more of the acetone bodies can be demonstrated in the urine in certain stages of the intoxication than can be accounted for by the protein destruction.

As regards the fats, experience teaches that not only would butyric acid produce the compounds in question, but that other higher fatty acids, if possessed of an even number of carbons, may also cause their formation. Further, laboratory experimentation has demonstrated that in the oxidation of fatty acids of large molecular weight, the molecules are progressively diminished by the loss of two carbons with each step in the decomposition. From this, it may be inferred that while both the protein and the fat are potentially sources of the acetone bodies, the fat seems to be the more probable source. The final solution of this phase of the problem, however, must be regarded as still in abeyance.

Differing from the group of acid intoxications, in which imperfect oxidation seems to be the causative factor, and in which the chief chemical features are the diminution of the alkalinity of the blood, and consequently of the alveolar carbonic acid, together with the elimination by the kidneys of the acetone bodies, and a complementary increase of the ammonia in the urine, is a group of pathological conditions, marked also by an acid intoxication, but in which functional disturbance of the liver seems to be the

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dominant cause. The intoxications by phosphorus and chloroform, the general toxemias of pregnancy, cyclic, pernicious vomiting, and acute yellow atrophy, are among the conditions falling in this second classification. Here, the acid is not of the group already mentioned, but is sarcolactic acid. This substance eliminated by the kidneys appears in the urine in liberal quantities, and associated with it is a marked increase, both in the ammonia content of the urine, and also that of the amino acids. That there is usually an accompanying diminution in the urea, points definitely to an impairment of the urea forming function of the liver, the ammonia and amino acids representing precursors of this final end product of general protein metabolism. In certain conditions, notably in the toxemias of pregnancy, there is, in addition to the lactic acid and the ammonia increase, an increase of the acetone bodies, so that the ammonia may be regarded as of two-fold origin. This is further borne out by the fact that the increase in ammonia is greater in the pernicious vomiting associated with pregnancy, than in eclampsia. That the latter is to be regarded more essentially as a toxemia, resting in part on disturbed liver function, while, in the former, imperfect oxidation also plays a significant role, is in perfect harmony with the facts presented above. Finally, the fact has been frequently observed that in what may be called normal pregnancy the organism seems to have lost, in part at least,

its power of toleration of a carbohydrate deficiency. That the acidosis of the acetone bodies always causes a lessening of the alveolar carbon dioxide, and that the pernicious vomiting of pregnancy, in which this is a factor, is associated both with the non-convulsive toxemias and also with eclampsia, implies that the determination of the carbon dioxide elimination by the lungs should be of potential value, both from the diagnostic and the prognostic standpoint. Similarly, the determination of urinary ammonia, associated as it is with these conditions, is also to be regarded as of potential value in indicating the earlier stages of these types of acid intoxication. The chemical aspects of the determination of both ammonia and of carbon dioxide are varied and are affected by the imposition of certain limiting conditions. If the assumption may be made that the determination of one or the other of these is of sufficient value to justify its performance as a routine diagnostic procedure, then the adoption of a method, or of methods, which will give a sufficient degree of accuracy with a minimum of time consumption and complexity of apparatus and equipment, becomes a suitable matter for study. Since in the practical experience of every day, such determinations can be but minor incidents in the daily work of the busy medical practitioner, a study to be applicable to the needs of

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practice must include not only the consideration of sensitiveness, dependability, and accuracy, but also of simplicity and of time consumption.

The study then resolves itself into

(1) a critical consideration of the various methods for the determination, respectively, of carbon dioxide in the alveolar air and of ammonia in the urine, leading to the selection of one or more methods for each which conform to the criteria established: and

(2) by these methods, as adopted, to determine the relative dependability of the one or the other factor as an indication of approaching or incipient intoxication. To this could be suitably added continuous studies in those cases in which the toxemia failed of inhibition through treatment, to determine which could be regarded as the more suitable for following the progress of the case and giving the greater information for the prognosis. With these points in mind, the various available methods may now be subjected to critical analysis.

A M M O N I A   D E T E R M I N A T I O N S .

AMMONIA DETERMINATIONS.

The methods of determination of urinary ammonia may be classified under two general headings: either it may be determined directly in the original solution by a wide variety of methods, or indirectly by removal in one way or another from the solution, the amount subsequently being estimated either by gravimetric, volumetric or colorimetric procedure.

The first course, determination in the original solution, offers many analytical possibilities; the ammonia nitrogen may be liberated as a gas by decomposition of its compounds and measured in suitable apparatus; other substances that might interfere with the colorimetric procedure may be removed and a method of direct Nesslerization and colorimetric determination employed; the ammonia may be precipitated by a suitable reagent in the form of a white cloud the amount being determined nephlometrically by comparison with known amounts similarly treated; or it may be determined indirectly by volumetric procedure according to the method best known as the Ronchese-Malfatti or formol titration.

The removal of the ammonia from the solution according to the second course, may likewise be accomplished in various ways. First, there is the aeration of the solution containing the ammonia compound the solution having been rendered strongly alkaline, (a) in the cold under atmospheric pressure, (b) the same, but under reduced pressure;

second, there are the complementary methods where the ammonia is expelled from its compounds by heating either under normal or reduced pressure; and third, there is the precipitation and removal of ammonia by the use of a reagent with which it enters into chemical combination and from which it is subsequently liberated. In the aeration and distillation procedures the ammonia is in each case fixed by some acid solution placed in the receiver and the amount absorbed is determined gravimetrically by the formation of ammonium chloroplatinate or acid tartrate, volumetrically by titration of the excess of standard acid, or by the development of color with the Nessler-Winkler reagent and comparison with color produced under similar technique with known amounts of ammonia. The amount of the ammonia removed by precipitation may also be determined colorimetrically.

The following table classifies the various methods in a manner which may make clearer the subsequent analysis and criticism.

Determination of Ammonia.

I. Direct In Original Solution.

1. Decomposition of Ammonia.
    a. Lematte
    b. Florence
    c. Bacon.

2. Direct Nesslerization.
    a. Folin and Denis.
    b. Sumner

3. Nephlometric
    a. Graves

4. Direct Titration.
    a. Ronchese-Malfatti

II. Indirect After Removal from Solution.

1. Vacuum Distillation.
    a. Boussingault
    b. Würster
    c. Nencki-Zaleski and Soldern
    d. Steyer
    e. Schaffer-Titration
    f. Krüger Reich Schittenhelm
2. Distillation.
    a. Schlösing-Titration
    b. deGraaff-Titration
    c. Bonnema-Titration
    d. Winkler-Titration
3. Aeration.
    a. Folin-Titration
    b. Kober-Titration
    c. Davis
    d. Folin and MaCallum Titration or Colorimeter
4. Precipitation.
    a. Folin and Bell-Colorimeter.

The determination of ammonia by decomposition according to the methods outlined by Lematte (1), Florence (2) and Bacon (3), is at best indirect. The methods involve the determination of urea plus ammonia and the determination of urea alone, the ammonia value being determined by difference. Lematte precipitates the ammonia salts with phosphotungstic acid and magnesium chloride and determines the urea in the filtrate by the hypobromite method. Florence removes Uric acid and Creatinine with basic lead acetate and decomposes the ammonia salts by heating with the same reagent and determines the urea in resulting solution by the hypobromite method. Bacon uses Millon's reagent which yields Nitrogen and carbon dioxide in equal volume but does not decompose ammonia or its salts. This reaction like the hypo-

1. L. Lematte Comp. Rend. Soc. de Biol. (1913)
2. A. Florence " " " " " (1910)
3. R.F.Bacon Phil. Jr. of Science. (1909)

bromite of the other two is not quantitative, approximate results only being obtained. According to all three procedures the urea plus ammonia value is determined by means of hypobromite methods. The basis of the various hypobromite methods depends upon the fact that urea and ammonia-containing compounds are decomposed by a solution of sodium hypobromite with the formation of carbon dioxide and gaseous nitrogen. The carbon dioxide is retained by the excess alkali in the solution and the nitrogen gas is collected and measured. The method when carried out with the greatest of care, avoiding all possible mechanical errors due to effects of temperature, lack of alkali or reagent, loss of gas, and incorrect graduation of the apparatus, is at best but a clinical method for the estimation of urea and ammonia nitrogen, and is in no sense a strictly quantitative determination. Robinson and Muller have made comparative studies of the method using the Doremus-Hinds Ureometer and comparing it with the Folin-Pettibone Phosphoric acid method. Their conclusions are that the method gives inconsistent and completely unreliable results when performed in the usual way as described in textbooks, a constant reading not being obtained for from five to twenty-four hours after application of the reagent. They also find that uncorrected instruments give readings which vary from ninety-seven to one hundred and five percent of the correct value. In the presence of glucose in the urines there is an apparent catalysis and the readings are much higher than those of normal urines.

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Since the ammonia content is determined by difference, the errors incident to the two reactions exercise the maximum influence upon its absolute magnitude. Further, as under the most favorable conditions the ammonia nitrogen is but a relatively small fraction of the total amount determined, the error not infrequently assumes wholly vitiating proportions.

The methods for direct Nesslerization are two; the Merck's blood charcoal method suggested by Folin and Denis(1), and the copper hydroxide method described by Sumner (2). The first method depends upon the use of blood charcoal to remove by adsorbtion the greater part of the creatinine, Uric acid and pigmentary bodies, all of which would interfere with the colorimetric determination. The procedure is in brief as follows:
To 10cc of Urine in a flask is added 1cc of 25% m-phosphoric acid, 9cc distilled water and 2gm. of blood charcoal. Shake well for at least one minute and filter thro a dry paper. Transfer 1 to 5cc of the filtrate to a 100cc volumetric flask, add distilled water to 70cc Nesslerize by the addition of 15cc Nessler's solution, make up to volume with distilled water and compare with a solution obtained by Nesslerizing 1 mg. of ammonia nitrogen in another 100cc flask. The method is rapid and reasonably accurate. As used by its authors it gives results which

1. O. Folin and W. Denis Jr. of Biol. Chem. (1916)
2. J.B.Sumner " " " " (1918)

are comparable with those obtained by the Folin-MaCallum macro-aeration method. The accuracy of the method, however, is dependent upon the efficiency of the reagent which is a highly variable quantity. Moreover, none but Merck's purified blood charcoal has been found to have the necessary properties, and that reagent is not made in this country and cannot be obtained.

In the second method cupric hydroxide is made use of to precipitate the creatinine. The technique of the method is as follows: Pipette into a large test tube 10cc saturated copper sulphate solution, 15cc of Urine and 10cc of sodium hydroxide (Normality 2.03). Stopper. Shake. Filter, covering the funnel to prevent evaporation. Nesslerize a portion of the filtrate and compare in the colorimeter against a standard containing one milligram of nitrogen. The author's tabulated results show the method to be comparable with the aeration procedure. The sodium hydroxide solution should be free both from iron and carbonate and the copper sulphate must be free from ammonia. Too, in either an acid or an alkaline medium the precipitation of both creatinine and copper is interfered with. At the neutral point 90% of the Creatinine will be carried down.

The Nephlometric determination as suggested by Graves (1) as a substitute for the colorimetric determination with the Nessler-Winkler reagent is a relatively unimportant method. It requires the use of apparatus that is not commonly found in the laboratory and offers no ad-

1. S. S. Graves Jr. of the Amer. Chem. Soc. (1915)

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1. S. S. Graves. Jr. of the Amer. Chem. Soc. (1915)

vantages over the colorimetric method. He suggests the precipitation of ammonia from the solution by means of a reagent prepared from 50cc $HgCl_2$, 15 gm NaCl, 35cc saturated Li_2CO_3 solution and 65cc of water, and its subsequent comparison with similarly precipitated known amounts of ammonia.

The last of these direct determinations in the original solution is the Ronchese-Malfatti (1) formalin titration which offers excellent possibilities, and while the general concensus of opinion places it among the relatively inaccurate methods, a careful analysis of the probable sources of error shows that several of these may readily be corrected. This permits of an accuracy with which the method has never been credited. The method may be briefly described as follows:

A standard sample of the urine, usually 25 cubic centimeters, is rendered exactly neutral to phenolphthalein by the addition of tenth-normal sodium hydroxide. Diluting the urine with distilled water sharpens the end point, and the addition of solid potassium oxalate, as suggested by Folin, eliminates the disturbing effect of the calcium salts. To the neutral urine is next added a definite amount of neutral commercial formaldehyde solution. It is imperative that the neutrality of this latter be insured, as the commercial product always contains appreciable amounts of formic acid, and the auto-oxidative process goes

1. A. Ronchese Comp. Rend. Soc. de Biol. (1907)
2. H. Malfatti Zeit. f. Anal. Chem. (1908)

on whenever the solution is exposed to the air. To the neutral solution thus obtained, tenth-normal caustic soda is run in until the appearance of the pink color due to the phenolphthalein shows that the neutral point has been attained. The method depends upon the fact that the caustic soda liberates ammonia from its several compounds, and that the latter, in the presence of formaldehyde, reacts quantitatively to form hexamethylenetetramine, a compound which is neutral toward phenolphthalein. The volume of caustic soda determines at once the amount of ammonia present.

One source of error in this method rests upon the fact that amino groups show the same reaction as do ammonia compounds. Under ordinary conditions, the amino acid content of the urine is so small as to make unlikely their forming a disturbing factor in the method. In, however, conditions of functional impairment of the liver, where the deaminizing power of the organ is impaired, the amino acid content of the urine increases to appreciable proportions, and thus forms a serious source of error in the direct ammonia determination. In consideration of the fact that the ammonia increase is, in part, due to the same cause as that which increased the amino acids: namely, an impairment of the urea-genetic function, the significance of the one compound is essentially that of the other. The significance of a marked increase in either or both would be the same. For the purposes of this study, then, this amino acid error becomes relatively insignificant.

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Another source of error, which seems to have been ignored in the literature, but which offers an absolute and variable element of uncertainty, lies in the carbonate content of the caustic soda solution, and of the urine. Since phenolphthalein, a substance easily affected by carbonates has been selected as the indicator, it is obvious that unless this factor can be eliminated, the possible error of the method is increased. The alkali should be prepared in the carbonate-free state, and then maintained free from carbon dioxide contamination. The assumption that high-grade, alcohol-purified sodium hydroxide is free from carbonate contamination may be demonstrated to be erroneous. The best grades of caustic soda on the market will show from two to five percent of carbonate. If to this is added the carbon dioxide contained in the average laboratory supply of distilled water, the amount is further increased. The procedure followed in this laboratory is as follows:

The caustic soda solution is prepared at slightly greater strength than in the final concentration desired. The carbonate content is determined by parallel titrations at zero degree, using respectively phenolphthalein and methyl orange as indicators. Then slightly more barium hydroxide than the calculated amount necessary to precipitate the carbonate is added. After the carbonate precipitate has settled, the clear liquid is decanted in a carbon dioxide-free atmosphere, and the barium content determined

gravimetrically as barium sulphate. The calculated amount of sulphuric acid, or better sodium sulphate, is added, and after the subsidence of the barium sulphate precipitate, the carbonate and barium-free alkali solution is transferred in a carbon dioxide-free atmosphere to a paraffin-lined stock bottle. The use of absorbing towers filled with soda lime for the purification of all air entering the stock bottle maintains the liquid free from carbonate contamination.

The second source of carbonate contamination: namely, the carbon dioxide in the urine, can be readily eliminated by the method of procedure. The initial neutralization of the urine is affected by the carbonate content, thereby introducing an error into the determination of the titratable acidity, which constitutes this initial step. This fact has not been touched upon in the literature, so far as the author is aware, and experiments made in this laboratory show that errors of appreciable magnitude may arise in this connection. The urine being neutral toward phenolphthalein after the completion of this step, the second titration merely involves a re-establishment of the same P_h value, and if the alkali be free from carbonate the amount used is a measure of the ammonia content.

When we come to the indirect determination of ammonia the first problem is the liberation of ammonia from its compounds in the urine without causing the decomposition of other nitrogen containing substances present

there. Various alkalis have been tried. Milk of lime and magnesia were used in the early methods of Schlösing (1) Boussingault (2) and Würster (3). The lime gives off the ammonia rapidly but causes the decomposition of other substances resulting in very high ammonia values. The magnesia on the other hand gives off the ammonia very slowly and the removal is incomplete, due to the formation of ammonium-magnesium phosphate crystals from which the ammonia cannot be liberated by magnesia. Boussingault also made use of sodium carbonate the reagent which has since been suggested by Folin (4) and Schaffer (5). This reagent drives off the ammonia more rapidly than does the magnesia and does not cause decomposition as the lime does. It has, however, been shown by Steele (6) and by S. R. Benedict (7) that in the presence of magnesium in the urine, the addition of carbonate and the process of aeration will cause the formation of ammonium-magnesium phosphate crystals. Steele and Gies (8) suggest the use of sodium hydroxide for the purpose of liberating the ammonia and the addition of sodium chloride to force back the dissociation of the hydroxide and render its action less drastic. The experimental evidence offered by both Steele and Benedict demonstrates

1. Schlösing Annales de Chem. et de Phys. (1851)
2. Boussingault " " " " " " (1850)
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7. S. R. Benedict and Emil Osterberg, Biochem. Bull. (1913)
8. Steele and Gies, Jr. Biol. Chem. (1909)

that this reagent will liberate ammonia quantitatively from the triple phosphate and that other substances are not decomposed by the treatment so as to yield ammonia. Folin (1) preferring sodium carbonate to sodium hydroxide modified his method by adding acid to dissolve the crystals and from seven to eleven grams of potassium oxalate to the sodium carbonate. This modification Benedict finds is not so satisfactory in the original Folin method as is the modification by Steele and Gies, tho in the macro-aeration method where the amount of oxalate necessary is decreased, the method is successful. Potassium hydroxide cannot be used to liberate the ammonia, for potassium sulphate if formed may carry down ammonia by occlusion as a double salt.

The ammonia driven out of solution in all distillation and aeration procedures is fixed by some acid solution placed in the receiver. The amount of ammonia is then determined by titration of the excess acid, by the development of color with the Nessler-Winkler reagent or as suggested by Foxwell (2) with phenol and sodium chlorate, or gravimetrically by the preparation of an insoluble ammonium salt as chloroplatinate or acid tartrate. The acid used in the collection is usually sulphuric; hydrochloric, however, is used by Folin and McCallum (3) in their aeration procedure and Winkler modifies the Kjeldahl procedure by the use of Boric acid. The relative merits of titration and colorimetric determinations are still a matter of controversy among the advocates of one or the other procedure.

1. O. Folin, Jr. Biol. Chem. (1910)
2. Foxwell, Gas World, (1916)
3. O. Folin and MacCallum, Jr. Biol. Chem. (1912)

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1. O. Folin, Jr. Biol. Chem. (1910)
2. Foxwell, Gas World, (1915)

The matter will be discussed further in connection with the methods involving their use.

The colorimetric method as suggested by Foxwell, has not, so far as the writer knows, been applied to urinary determinations. He treats the ammonia solution with phenol and sodium chlorate and compares it in a small colorimeter, such as is used in the Marriott carbon dioxide determination, with previously prepared standard solutions containing varying amounts of ammonia similarly treated with phenol and sodium chlorate. The method is potentially useful as a clinical procedure in that it obviates the necessity of the purchase of an expensive colorimeter which of course is necessary for the Nessler-Winkler comparisons. The length of time that the standards prepared for the Foxwell comparison would retain their integrity, is a factor that would enter into the evaluation of the method.

The gravimetric determination is susceptible of certain unavoidable errors which when linked to the elaborate and time consuming technique of the method makes it obviously unsuited for the problem under consideration.

Vacuum distillation is, so far as the writer can find, the earliest method used for the removal of ammonia from the urine. In 1850 Boussingault published a method for determining ammonia by distilling to dryness with milk of lime in a vacuum giving 40 to 50 degrees Centigrade. The operation took about an hour. This, tho undoubtedly the original application of vacuum distillation to the determination of ammonia, appears to have received little attention. Würster in 1887 proposed a similar method for

distillation and apparently was not aware of Boussingault's work. Würster (1) distilled at fifteen milimeters pressure at 50 degrees Centigrade using five, ten or twenty cc of Urine with baryta water, magnesia or lime, and claimed the operation to be completed after five, ten or fifteen minutes depending upon the amount of urine used. The method is inaccurate due to the small quantities of urine used and to the incompleteness of the liberation of ammonia under the conditions of the determination. The results while acceptable in the last century, are not to be compared with the accuracy of present day methods. The apparatus used was bulky and inconvenient. Modifications of the method were made by Nencki (2), Zaleski (2) and Soldern (3) and by Steyer (4), modifications all of which sought to construct an apparatus by various arrangements of flasks which would obviate the loss of the determination by the foam getting into the alkali. The modifications increased the accuracy also by increasing the quantity of urine and continuing the distillation over a longer period of time.

In 1903 Schaffer (5) made a modification of the original Boussingault method. In order to shorten the period of distillation and so lessen the error due to decomposition without using too small amounts of the urine Schaffer added methyl alcohol to the urine thus lowering

1. C. Würster, Central. f. Phys. (1887)
2. M. Nencki and J. Zaleski, Archiv. f. Exper. Path. (1895)
3. Soldern, Zeit f. Biol. (1899)
4. Steyer, Beitr. zur Chem. Phys. u. Path. (1902)
5. Schaffer, Am. Jr. of Phys. (1903)

the boiling point so that a more rapid ebullition would take place. He reduced the time to fifteen minutes, using sodium carbònate to liberate the ammonia. In the same year, Kruger, Reich and Schittenhelm (1) published a similar method using sodium carbonate and sodium chloride and added ethyl alcohol to prevent foaming.

The method of Schaffer may be considered as typical of this type of determination. It is in brief as follows: To 50cc of urine in a flask, add an excess of sodium chloride and about 50cc of methyl alcohol. This is connected with two absorption flasks in series, the first containing 50cc, the second 10cc of standard tenth normal acid. The second bottle is in turn connected through a safety flask with a vacuum pump. The urine container is placed in a water bath at 50^{o} and one gram of dry sodium carbonate added; suction is then started and the boiling continued for fifteen minutes. The acid in the two absorption flasks is then titrated, using Alizarin as the indicator.

A distillation method without the use of a vacuum was described by Schlösing (2) in 1851 and was used to determine the ammonia in tobacco. In 1898 Neubauer (3) in his "Anteilung für Harn Analyse" applied the method to urine. As described by Neubauer the method was as follows: To 10cc of urine was added magnesia or milk of lime and this mixture was placed in a flat dish ten or twelve centimeters wide; over

1. Kruger, Reich and Schittenhelm, Jr. f. Phys. Chem. (1903)
2. Schlösing, Annal. de. Chem. et de Phys. (1851)
3. Neubauer, Jr. f. Prak. Chem. (1855)

this dish and supported by a triangle was another dish and supported by a triangle was another dish containing the acid; all was closed by a tightly fitted bell jar and allowed to stand forty-eight hours. The authors used standard acids and alkali of such strength as to make it impossible to measure the small quantities of ammonia given off on standing longer than forty-eight hours and so considered their determinations complete. The method is susceptible of many errors and is decidedly unreliable.

Heat distillations using modifications of the Kjeldahl apparatus have been suggested by other chemists. De Graaff and Bonnema (1) distil into N/10 acid using sodium carbonate and calcium oxide respectively to liberate the ammonia. Ethyl alcohol is added to prevent foaming. Winkler uses Boric acid for the absorption of the ammonia and titrates with standard hydrochloric or sulphuric acid using Congo red or Methyl orange as indicators. The method is of necessity not an exact one. Boric acid is too weak an acid to completely absorb the ammonia and the end point in a titration with either Congo red or Methyl orange is not sharp.

Folin in 1903 worked out the first of the aeration methods as a substitute for distillation. The procedure in brief is as follows: Place 25cc of urine in an aerometer cylinder, add about 1 gm. of dry sodium carbonate and introduce some crude petroleum to prevent foaming. Connect with the suction pump through a cal-

1.	DeGraaff,	Pharm. Weekblad	(1916)
	Bonnema,	Chem. Ztg.	(1915)

cium chloride tube filled with cotton and an absorbing flask containing 20cc of N/10 sulphuric acid, ammonia free water and a few drops of alizarin. With an ordinary filter pump and good water pressure the last trace of ammonia should be removed in about an hour and a half. The number of cubic centimeters of the N/10 acid neutralized by the ammonia of the urine may be determined by direct titration with N/10 sodium hydroxide. The modification in the use of a reagent suggested by Steele has already been discussed. Folin recommends the aeration procedure as a substitute for Kjeldahl nitrogen determination. Whether or not it is an acceptable substitute has been the subject of some controversy. If there is an adequate volume of air, a small volume of liquid and an excess of alkali, the method is susceptible of a high degree of accuracy. Kober (1) in 1909 and Davis (2) in 1909 describe aeration methods similar in technique to that of Folin.

The Folin-MaCallum (3) micro-aeration method which is a modification of the original Folin is a combination of the aeration procedure for ammonia with its colorimetric determination by means of the Nessler-Winkler reagent. Because small quantities of urine are used, thus shortening the time necessary for aeration, the method is considered by its authors available for clinical determinations. From one to five cc of the urine are placed in a test-tube, a few drops of a solution of potassium carbonate and potassium

1. P.A.Kober, Jr. Amer. Chem. Soc. (1913)
2. R.O.Davis, " " " " (1909)
3. Folin and MaCallum, Jr. Biol. Chem. (1912)

oxalate are added, and a strong air current is passed through the mixture until the ammonia has all been removed. The ammonia is collected in ammonia-free water and acid, is Nesslerized, and the amounts determined by comparison with a standard ammonium sulphate solution similarly Nesslerized.

The last and latest of the indirect methods for the determination of ammonia is the so-called permutit method suggested by Folin and Bell.(1) The reagent which is sold under the trade name of "Permutit" is a complex insoluble sodium salt containing easily replaceable sodium. The chemical basis of the method is the absorption of ammonia by this reagent, its subsequent liberation by alkali and determination by Nesslerization. The accuracy of the method is dependent entirely upon the quality of the reagent, the amount liberated depending upon this variable factor.

1. Folin and Bell. Jr. Biol. Chem. (1917)

C A R B O N D I O X I D E D E T E R M I N A T I O N .

CARBON DIOXIDE DETERMINATION.

The determination of carbon dioxide as a constituent of the respiratory gases may be accomplished by numerous and diverse procedures. These may be grouped under three heads:

first, the analysis of the gases thro one or another gas analytical procedure;

second, the fixation of the carbon dioxide by some chemical agent and the determination of the material thus formed either by gravimetric, volumetric, colorimetric or thermometric operations,

and third, the optical determination which is dependent upon interference phenomena. Further, each of the first two general types of analyses differ in that certain methods proposed, offer a maximum of accuracy with a minimum of simplicity and a maximum of time consumption, while others show the exact opposite of the first.

While our purpose in this work is to secure a method which will offer a sufficient degree of accuracy with a minimum of time consumption, complexity of apparatus and equipment, still in the preliminary work one accurate method must be adopted in order to create a standard of comparison for the less accurate and simpler methods. The following classification will give some idea of the methods available for carbon dioxide determination.

I. Determination by Gas Analysis.

Winkler Gas Burette

A. Measurement and Absorption in same burette.

1. Hempel's modified Winkler Burette.
2. Honigman Gas Burette.
3. Bunte Gas Burette.
4. Fridericia collection, measuring and absorption tube.

B. Measurement and Absorption in separate apparatus.

1. Hempel gas apparatus.
2. Elliot gas apparatus.
3. Orsat gas apparatus.
4. Bunsen gas apparatus.
5. Doyere gas apparatus.
6. Herbert and Heim gas apparatus.

C. Measurement and Absorption separate with the addition of means for compensating changes in temperature and barometric pressure.

1. Pettersson gas apparatus.
2. Hempel gas apparatus.
3. Pettersson and Palmqvist gas apparatus.
4. Haldane gas apparatus.
5. Henderson gas apparatus.

D. Change of density with changing chemical composition.

1. Nernst Gas Balance.

II. Determination after the Fixation of the Carbon Dioxide.

A. Gravimetric.

1. Haldane Carbon dioxide absorber.
2. Benedict " "

B. Volumetric.

1. Saussare and Pettenkofer titration.
2. Hesse titration.
3. Henderson titration.

C. Colorimetric.

1. Marriott determination.

D. Thermometric.

1. Wilcox thermometer.
2. Munroe thermometer.

III. Determination with interferometer.

The various types of methods will be considered in order of classification with an estimation of their applicability to the determination of alveolar carbon dioxide, and of their availability for use in the present problem. The methods for the collection of the alveolar air, which is of course pre-requisite to all procedures for carbon dioxide determination involved in this study, are an important phase of the subject and will be subsequently considered. For the time being we will consider that the gas is available for the determination, in one manner or another, of its carbon dioxide content.

Measurement of the amount of the gas is the first operation in the use of a gas analytical method. Such measurement is best accomplished by the determination of the volume of the gas under known conditions of temperature, of pressure and of tension of liquids that are present. The earliest and simplest apparatus for gas measurement is the Winkler Gas Burette (1), commonly called the Hempel Gas Burette. As a matter of fact, however, Hempel's original burette is a modification of the Winkler. This Winkler Burette consists of two tubes A and B which are set in iron feet. Inside the feet the tubes are bent at right angles and conically drawn out, the ends projecting from the feet. The two tubes are connected by rubber tubing slipped over these projections. The measuring tube A ends at the top in a capillary tube over which a short piece of rubber tubing closed with a pinchcock

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is slipped. A contains between the base of the capillary and a graduation a little above the iron foot one hundred cubic centimeters and is graduated to fifths of a cubic centimeter. B is used as a leveling tube and as a means of introducing absorbing solutions into the measuring burette. Manipulation of this first apparatus must have been something of an ordeal, and would require a high degree of technique to secure results even approximating those obtained by present day methods.

The procedure was as follows:

Fill the tubes A and B with water, taking care to drive all air out of the connecting rubber tube by raising or lowering the tubes. Then, join the burette to the vessel containing the gas by means of a glass or rubber tube filled with water. To fill the burette with the gas to be examined, close the connecting tube between A and B and pour the water out of B. Place the leveling tube on the floor and open the pinchcock at the top of A. The water will flow into the leveling-tube and the gas will be drawn into the burette. When A is filled with gas close the pinchcock, disconnect from the gas-holder and after the liquid has run down from the walls of the burette, take up the tubes by the iron feet and by raising or lowering bring the water in the tubes to the same level. The gas is now under atmospheric pressure, and its volume is read off. To bring as much as possible of the absorbent into the burette, lower the leveling-tube until the expanded gas begins

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to enter the rubber tube, close the connecting tube and pour the greater part of the water out of B. The absorbing liquid is now poured into B and the tube raised as far as the rubber tube permits; in this way a considerable amount of the reagent, diluted with the water in the rubber tube is brought into the burette. Closing the tube at the base of A, bring the gas into close contact with the absorbent by vigorously shaking the burette. When no further decrease of the volume of the enclosed gas takes place, the reading is made as before. The difference in volume gives the amount of the absorbed gas.

Because of its solubility in water, the initial volume of a gas containing carbon dioxide cannot be made over water. Hempel therefore modified the original Winkler Burette. He added a three way cock at the bottom of the measuring burette. The burette is filled by drawing the gas thro the tube by means of suction applied at the lower cock and then closing both ends. Atmospheric pressure is obtained by opening the upper end momentarily. The absorbing liquids are introduced by means of a funnel connected with the longitudinal opening of the three way cock, by a piece of rubber tubing. Except for this the manipulation is the same as is described for the simple Winkler Burette.

This method of Winkler and its modification by Hempel are the basis not only of gas analysis involving separate absorption pipettes, as the various methods of Hempel, Orsat and their followers, but also of the Honigman, Bunte and Fridericia methods in which, as in the Winkler, the absorption takes place within the measuring

burette. These latter methods we will consider first.

Honigmann (1) did away with the leveling tube entirely, using for his determination simply a one hundred cubic centimeter burette closed at the top with a glass stopcock, the lower end being drawn out to smaller diameter to permit of a piece of rubber tubing being easily slipped over it. The absorbing liquid is placed in a very tall glass cylinder. The gas to be analyzed is passed thro the burette until all the air previously contained has been displaced. The stopcock is then closed and the rubber tube is immersed in a solution of potassium hydroxide. The burette is then lowered into the solution until the surrounding liquid stands at the zero point, the lowest graduation upon the tube, the stopcock is opened and the liquid allowed to rise inside the burette to the same mark. The tube now contains one hundred cubic centimeters of the gas at atmospheric pressure. The absorption of the gas is effected by inverting the burette and allowing the alkali to flow along the walls of the burette. The end of the rubber tube must of course remain below the surface of the liquid in the cylinder. After absorption the burette is lowered in the solution until the liquid surfaces within and without stand at the same height. The percentage of carbon dioxide present is read directly. The Honigmann burette is suited only to a rapid approximate determination of carbon dioxide in gas mixtures which contain fairly high percentages of that constituent.

1. Hempel's Method of Air Analysis. (1906)

The Bunte (1) apparatus consists of a burette, a suction bottle and a beaker of alkali. The burette is bulbed to avoid extreme length and is provided at the top with a funnel and a three-way cock and at the bottom with an ordinary cock. The gas is collected over water as in the original Winkler, the suction bottle taking the place of the leveling tube. To introduce the reagent the lower tip is immersed in the alkali and the lower cock opened. After the absorption of the carbon dioxide the reagent is washed from the burette by water introduced thro the funnel, the tip of the burette being all the time under water. Water then rises in the burette, filling the vacuum created by the removal of the carbon dioxide and the reading is made. The difference between the first and second readings represents the number of cubic centimeters of carbon dioxide present. Readings are made with the funnel filled to the same level and the upper cock open; the pressure being thus atmospheric pressure plus the pressure of the height of the column of water in the funnel. Determinations made with this apparatus are of necessity inaccurate for the gas is brought into contact with large volumes of water which are unsaturated with the gas mixture and will, therefore, absorb some of the gas of the sample. Reagent is also wasted because of its dilution with wash water. The use of the burette is like the Honigmann; limited to the approximate determination of carbon dioxide.

1. Hempel's Methods of Air Analysis. (1906)

The most recent development of the Winkler Gas burette along this line where the absorption and measurement are made in one tube, is the Fridericia (1) tube. This Fridericia tube and the method of its use are particularly interesting to us in this study, for it was devised by Fridericia and has been recommended by many others as a suitable method for the clinical determination of alveolar carbon dioxide. The apparatus necessary for the determination consists of the Fridericia absorbing pipette, in which the air is collected and analyzed, a cylinder of water at room temperature in which the tube may be immersed, and a five percent solution of caustic soda. The absorbing pipette is essentially a U-shaped tube with stopcocks so arranged, that it may be opened from end to end, allowing the air to be blown through, or closed so that one chamber of exactly one hundred cubic centimeters capacity may be closed off from the rest of the tube. The lower section of this chamber is graduated in tenths of one percent of the total volume of the chamber.

The method of procedure is as follows: At the end of a normal inspiration the subject is asked to blow through the open tube, emptying the lungs as far as possible. The upper end of the measured portion of the tube is closed and the air is cooled to room temperature by immersion in the tank, contraction within the measuring space being compensated by air from the open arm, the assumption being made that its composition is similar to that within the measuring space. How far diffusion vitiates this assumption has not been determined. Alkali is introduced into the

1. S. Fridericia, Munch. Med. Wochen. (1914)

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air chamber and the carbon dioxide is absorbed by it. The tube is returned to the tank, the lower cock is opened, and the carbon dioxide containing alkali is allowed to drain out; the water then rises in the tube, filling the vacuum created by the removal of the carbon dioxide. The percentage of carbon dioxide is read directly. This percentage as any of the percentages obtained by a gas analysis procedure may be changed to milimeters of mercury pressure in dry air by multiplying the difference between barometric pressure at the time of the test and the tension of aqueous vapor at 37.5°C which is 48 mm. mercury. The advantages of this method include its simplicity of design and of technique required for its use combined with the possibility of easy transportation of the apparatus to the bedside.

Because of the difficulties of manipulation and the need of cleaning the apparatus between each determination where the measurement and absorption is carried out in the same tube, the original gas burette was further modified by the addition of separate absorption apparatus. These were connected with the measuring burette by capillary tubes of as short length as possible, the air being forced over into the absorbers by the raising and lowering of the leveling tube. The error caused by measurement over water, the carbon dioxide being very soluble in water, was obviated by the use of mercury in the leveling tube. A drop of water placed in the measuring burette insures saturation of the gas. Further, the measuring burette was immersed in a water jacket thereby preventing changes in the gas volume due to

variations in temperature. The types of apparatus designed by Hempel (1), Elliott (2), Orsat (3) and many others show one or all of these modifications.

The third division in the classification of gas analytical methods, contains the latest descendants of the original Winkler burette. Their most important common feature is the use of a tube so connected with the measuring burette as to compensate for changes in atmospheric pressure. The possibility of such compensation was first suggested by Pettersson. The later apparatus of Hempel (4), the Pettersson-Palmqvist (5) apparatus and types designed by Haldane (6) in England and Henderson (7) and his co-workers in this country, all make use of a compensation tube. The Haldane portable apparatus offers a means of exact gas analysis, as is amply attested by the work of the many investigators making use of it. It has been adopted as the standard of comparison in this work, and description of it and the technique of its manipulation will serve to illustrate the methods employed in modern gas analysis.

The apparatus and method of use are fully described by Haldane in his brief treatise on "Methods of Air Analysis". In brief the procedure is as follows: The gas to be analyzed is drawn into a measuring burette

1 and 4.	Denis	Gas Analysis.	(1913)
2.	Elliott Scott,	Standard Methods of Chemical Analysis.	
3.	Orsat.	Chemical News.	(1874)
5.	Pettersson.	Zeit. f. Anal. Chem.	(1886)
6.	Haldane.	Methods of Gas Anal.	(1912)
7.	Henderson.	Jr. Biol. Chem. 38	(1918)

surrounded by a water jacket and is there saturated with water over mercury, cooled to room temperature and measured. In the same water jacket is a control tube of about the same volume as the burette. The measuring tube and the control tube are connected through the potash absorption pipette which serves as a pressure guage. Before every reading of the burette the potash is brought to the same level. The air in the burette is freed from carbon dioxide by forcing it back and forth into the absorption chamber containing potassium hydroxide. Measurement of the air is made before and after the operation and the percentage of carbon dioxide calculated. The method is one which gives a very high degree of accuracy with a minimum of time consumption and requires only a fair degree of technical skill.

The fourth group of methods, namely that depending upon varying densities of gas mixtures (1) with changing composition such as the gas balance, are all continuous procedures and all wholly unsuited for intermittent analysis. As but one phase of the respiratory cycle is subjected to analysis, only static methods are applicable.

Gas analysis is not, however, the only means available for the determination of this gas. Gravimetric methods are accurate and valuable where the determination of large quantities of the gas is desired. It is the standard method for use with respiration calorimeters (2). The carbon dioxide is absorbed by granulated soda lime and the carbon dioxide determined by the difference in the

1. Nernst and Hunter. Zeit. für Physikal. Chem. (1905)
2. Carpenter. Carnegie Instit. Public 216 (1915)

weight of the soda lime before and after absorption. Such a method requires of course the removal of all water from the air before it enters the carbon dioxide absorber. The absorber consists generally of either a metal cylinder or a glass container filled with the soda lime and provided with air tight connections permitting of the introduction and withdrawal of the air. Such a method might conceivably be used for this work. The subject could breathe for a period of ten minutes or so through a system containing a small meter for measuring the air, a sulphuric acid container for removing the water and a carbon dioxide absorber. The carbon dioxide tension could then be determined by weighing the absorber, the amount being divided by the volume of expired air. The oxygen level is maintained by use of a tank of pure gas in controlled connection. Such apparatus as the Benedict portable respiration unit,(1) is designed for this and kindred purposes. The relatively high cost of an installation, together with the somewhat complicated manipulation precludes the general use of this apparatus and limits it to institutions where these conditions can be met.

Another possibility is the fixation of carbon dioxide by some liquid reagent and its titrimetric determination. The procedures available are those of Pettenkofer(2) Henderson (3) and Marriott (4). The first two are essentially the same. A measured volume of air is in each case treated

1. Carpenter. Carnegie Instit. Public. 216 (1915)
2. Pettenkofer. Am. Jr. Phys. (1912)
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3. Henderson. " " " (1912)
4. Marriott. Jr. Am. Med. Association (1916)

with an excess of barium hydroxide solution which quantitatively absorbs carbon dioxide forming insoluble barium carbonate. Phenolphthalein is added to the solution and the excess of barium hydroxide is titrated with hydrochloric acid. From the amount of alkali used to absorb the carbon dioxide the amount of the latter is calculated. Pettenkofer uses a flask of known capacity and fills it with the air to be analyzed by means of bellows, notes the pressure and temperature, adds by means of a pipette one hundred cubic centimeters of barium hydroxide solution, shakes vigorously, pours into a dry flask, and pipettes out twenty-five cubic centimeters which he titrates with the hydrochloric acid. The strength of the barium hydroxide in terms of acid being known, the amount and percent of carbon dioxide can be calculated at 0^{o}centigrade and 760 milimeters of pressure. The method of Henderson is much simpler and unquestionably more exact. His apparatus permits of the air being sucked directly into a measured pipette from the Haldane gas collection tube, where it is treated with the barium hydroxide. The alkali is then withdrawn into a flask, the precipitate allowed to settle and the titration carried out with hydrochloric acid. The value from the clinical standpoint of both of these methods is lessened by the fact that standardized alkali and acid are necessary for the determination.

The determination by the method described by Marriott while likewise dependent upon the hydrogen ion concentration varies widely from the two just described. The apparatus consists of eight test tubes, containing standard

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phosphate solutions, a standard bicarbonate solution, a small test tube, a glass tube drawn out to a capillary point and a box for color comparison. The method depends upon the fact that if a current of air containing carbon dioxide is passed through a solution of sodium carbonate or bicarbonate until the solution is saturated, the final solution will contain sodium bicarbonate and dissolved carbon dioxide. The reaction of such a solution will depend upon the relative ammounts of the alkaline bicarbonate and the acid carbon dioxide present. This, in turn, will depend on the tension of carbon dioxide in the air with which the mixture was saturated, and will be independent of the volume of air blown through it, provided saturation has been obtained. High tensions of carbon dioxide change the reaction of the solution toward the acid side. Low tensions have the reverse effect; hence, the reaction of such a solution is a measure of the tension of carbon dioxide in the air with which it has been saturated. The reaction of such a solution may be determined by adding to it an indicator such as phenolsulphonephthalein which shows over a considerable range of reaction definite color changes. A certain color indicates a certain reaction. Solutions of a given reaction may be prepared by mixing acid and alkaline phosphates in definite proportions. Such solutions according to Marriott may be kept unaltered for long periods of time and can be used as standards for comparison. The eight standards are so prepared as to show a hydrogen ion concentration equivalent to that shown by carbon dioxide tensions of 10,15,20,25,30,35,40 and 45 mili-

meters of mercury in saturated bicarbonate solution. Comparisons are made with a flat black metal box, backed with opal glass and containing holes of the standard and the unknown. For the analysis of a sample of air about two or three cubic centimeters of the standard bicarbonate solution are poured into a clean test tube of the same diameter as the standard tubes. Air collected in a rubber bag is then blown through the solution, by means of a glass tube drawn out to a capillary point, until the solution is saturated as shown by the fact that no further color change occurs. The tube is stoppered and the color immediately compared with that in the standard tubes. The method is a very rapid one, its value is dependent upon the ease or difficulty with which correct standards may be prepared, the length of time that they will maintain their integrity, and the ability of the operator to apprehend fine color distinctions.

While according to Marriott the standards for comparison are easily prepared and will retain their integrity for a long time, work which has already been done in connection with this research would indicate that such an assertion betrays an unwarranted degree of optimism on the part of its author. Marriott's directions for the preparation of the standards are as follows:

"Fifteenth molecular acid potassium phosphate: Of the pure recrystallized salt (KH_2PO_4), 9.078 gms. are dissolved in distilled water, 200cc of 0.01 percent phenolsulphonephthalein solution are added, and the whole is made

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up to one liter.

Fifteenth molecular alkaline sodium phosphate: The pure recrystallized salt ($Na_2HPO_4 12H_2O$) is exposed to the air for from ten days to two weeks, protected from the dust. Ten molecules of water of crystallization are given off and a salt of the formula $Na_2HPO_4 2H_2O$ is obtained. Of this salt 11.876 gms. are dissolved in water, 200cc of 0.01 percent. phenolsulphonephthalein solution are added, and the whole is made up to one liter.

Proportions in which solutions are mixed:

mm.	10	15	20	25	30	35	40	45
Potassium phosphate	17.8	25.2	31.0	35.7	40.5	45.0	47.0	50.2
Sodium phosphate	82.2	74.8	69.0	64.3	59.5	55.0	53.0	49.8

The standard alkali solution for absorption is prepared either by weighing out 0.530 gms. of dessicated sodium carbonate or by measuring accurately 100cc of tenth normal sodium hydroxide into a one liter volumetric flask. 200cc of 0.01 percent. phenolsulphonephthalein are added, and the whole is made up to the mark with distilled water. Carbon dioxide from a cylinder or from the lungs may be passed through this solution to convert the alkali into bicarbonate or the solution may simply be used as it is, as the alveolar air that will be blown through the solution subsequently will accomplish the same purpose.

Owing to the difficulty of estimating the amount of water of crystallization in the sodium phosphate salt

the preparation of a fifteenth molecular solution is not the simple matter that it seems. Standards prepared according to Marriott's direction have not given solutions that check with analyses made by the Haldane apparatus nor with each other. For the purposes of this research fifteenth molecular mono- and di-sodium phosphate solutions are prepared from molar solutions of pure phosphoric acid and carbonate free sodium hydroxide. These are then mixed in the proportions indicated above. Since the action of the alkali upon the glass of the tubes even in a short time causes a change in the hydrogen ion concentration of the standards, the solutions thus prepared are placed in paraffin lined delivery bottles from which the standard tubes may be filled as often as is necessary. These tubes are closed with rubber stoppers during the period of use.

Thermoscopic (1) (2) determinations of carbon dioxide are possible but save for the noting of such possibility are of little interest to us here. The method has not been applied to biological determinations. It depends upon the measurement of the rise of temperature of pulverized sodium hydroxide over which the air containing the carbon dioxide is passed. The percentage of carbon dioxide is registered directly on the Thermoscope scale.

Widely at variance with all methods previously described, but one which for institutional work at any rate, might prove of real value, is the determination by the use of the interferometer (3). The principle of the

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2. Am. Gas Light Journal 99 62 (1913)
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interferometer in its application to the composition of gases may be described briefly as follows: First, a simple optical device permits the passage of selected beams of light to a screen and the interference phenomena rendered visible. Where crest meets crest in successive superimposed waves of light, there is an intensification of the illumination; where crest meets trough there is complete interference and consequent extinction. Naturally, all gradations between these limits are possible. In the interferometer in question, the apparatus is constructed in duplicate so that two interference pictures are thrown on the screen, one directly above the other. The light passes through two small chambers, one associated with each spectrum. So long as the index of refraction of the medium filling these two chambers is identical, the pictures of the upper and lower are directly comparable. If, however, the index of refraction of one medium is altered, there is a shifting of the lines to the right or left, as the case may be. By a simple screw devise carrying a scale and vernier, this shift can be measured and thus differences in refraction index ascertained. To apply this to carbon dioxide determinations in alveolar air, it is only necessary to calibrate the instrument, using pure carbon dioxide-free air as the standard, and then adding to it successive portions of carbon dioxide, placing this second gas mixture in the lower comparison chamber, the pure air filling the upper, and then measur-

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ing the actual displacement for the varying concentrations. The method is said to give an accuracy equivalent to .05% of carbon dioxide.

METHODS OF
OBTAINING ALVEOLAR AIR.

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Two types of procedure are possible in the obtaining of samples of Alveolar air, giving, according to the procedure followed, air which is in approximate equilibrium with the carbon dioxide tension of the arterial blood in the pulmonary artery or air which is in approximate equilibrium with the carbon dioxide tension of the venous blood in the pulmonary capillaries. The former is obtained by a single forced expiration, the latter by repeated rebreathing of the air.

Several types of apparatus for the first method are available. Haldane (1) Lindhard and Krogh (2) have designed apparatus for the collection of single expirations. The Fridericia tube belongs, of course, to this group. Haldane's is perhaps the simplest. This method uses a piece of garden hose with a glass mouth piece at one end. The subject blows through the tube closing the mouthpiece with the tongue at the end of the expiration. The sample of air is drawn from the end of the tube. The other forms use valves for closing the tube, a necessary modification with untrained subjects. Experiments carried out by Haldane show that a true sample of Alveolar air can be obtained by this method. He observes, First: that the frequency of breathing may be varied within wide limits without altering the Alveolar Carbon Dioxide percentage, provided that the depth of breathing is allowed to regulate itself naturally, with no forcing or holding back.

1. Haldane. Am. Jr. Phys. (1915)
2. A. Krogh and J.Lindhard. Jr. Phys. (1914)

Respiration per minute.	Percentage of carbon dioxide.
30	5.67
4	5.66
36	5.68
3	5.71
24	5.48
6	5.56
4	5.72

Second: That when during normal breathing, air is expelled sharply from the lungs, the partial pressure of carbon dioxide in the expired air is constant after a certain volume has been expelled.

Depth of Expiration.	Percentage of Carbon dioxide.
190cc	3.03
335cc	4.37
510cc	5.04
650cc	5.19
950cc	5.51
1350cc	5.48

Also, he found that the mean of six successive determinations with a 900cc expiration showed a carbon dioxide percentage of 5.39 while a similar mean with an expiration of 1750cc showed a carbon dioxide percentage of 5.36. The deeper part of the expiration contains no more carbon dioxide than the middle part. Since the normal exchange of air is above 1000cc, it is evident that a fair sample of alveolar air is obtained by this method.

For the obtaining of samples of air by rebreathing, the Plesch method or one of its modifications is used. The apparatus and procedure used in Higgins' modification of the Plesch method is as follows: "To a copper disk of about 20cm. is soldered on the underside a flange 2cm wide. In the center of the same side of the disk is soldered an ordinary

3/4 inch three-way brass valve. Near this is soldered a small brass tap suitable for the attachment of small rubber tubing for taking samples of air. Over the top of the disk is stretched a rubber bathing cap, held in place by a large rubber band. The subject, with his nose clamped, breathes through a mouthpiece which is attached by a short piece of wide-bore rubber tubing to the three-way in the bottom of the copper disk. The tap is successively turned so that the subject first breathes to the outer air and then into the bag. After a definite amount of rebreathing, it is turned again, the bag is closed and a sample of the air is drawn from the bag for analysis." Different samples taken from the bag will show variations in the tension of carbon dioxide unless the air is thoroughly mixed by kneading the bag gently. More factors would seem to influence air obtained by this method than by that of Haldane. The tension of the carbon dioxide in the bag varies considerably with the depth of the initial inspiration and thus with the amount of air in the system of respiratory tract plus bag.

Moderately deep initial inspiration-
Carbon dioxide tension = 43.2 mm
Deeper initial inspiration-
Carbon dioxide tension = 41.5 mm
Very deep initial inspiration-
Carbon dioxide tension = 38.2 mm
Haldane (single expiration)
Carbon dioxide tension = 41.2 mm

This source of error can be to a degree avoided by adopting the suggestion of Higgins and filling the bag with a constant volume of air. The tap is turned at the end of a normal

expiration and the patient begins by inspiring from the bag. The time during which the breathing takes place is also a factor. After breathing from a bag four times in 20" the carbon dioxide tension was found to be 39.5 mm. After an interval of 3 1/2 minutes the same air was rebreathed for two periods of 20" each, the periods separated by an interval of one minute. The carbon dioxide tension then was 45.9 mm. After rebreathing for two more similar periods the carbon dioxide tension had risen to 46.4 mm. Continuation of the length of a single period will likewise cause a rise in the carbon dioxide tension.

4 respirations	in 20"	Carbon dioxide	= 45.2 mm
6 "	" 30"	" "	= 47.4 mm
8 "	" 40"	" "	= 48.6 mm

The rate and depth of respiration while rebreathing is still another factor.

5 respiration	in 25"	Moderately deep	-Carbon dioxide	=46.4 mm
16 "	" "	Shallow	" "	=45.9 mm
5 "	" "	Very deep	" "	=46.3 mm
17 "	" "	Shallow	" "	=45.4 mm
5 "	" "	Natural	" "	=39.0 mm
5 "	" "	Moderately deep	" "	=45.1 mm

With the exception of the fifth determination in which the subject was breathing naturally, quietly and superficially, the values of the carbon dioxide tension agree well, whether the respirations were very deep or were rapid and shallow. Gentle superficial respiration does not suffice to bring about complete diffusion of gases. The same has been found to be true of very rapid superficial, panting breathing which also gives too low values for the carbon dioxide tension. Respirations of moderate depth seem to bring about as complete diffusion as very deep respirations. Best results have been obtained

expiration and the patient begins by inspiring from the bag. The time during which the breathing takes place is also a factor. After breathing from a bag four times in 30" the carbon dioxide tension was found to be 39.5 mm. After an interval of 3 1/2 minutes the same air was rebreathed for two periods of 20" each, the periods separated by an interval of one minute. The carbon dioxide tension then was 45.9 mm. After rebreathing for two more similar periods the carbon dioxide tension had risen to 45.4 mm. Continuation of the length of a single period will likewise cause a rise in the carbon dioxide tension.

4	respirations	in	20"	Carbon	dioxide	= 45.2 mm
6	"	"	30"	"	"	= 47.1 mm
8	"	"	40"	"	"	= 52.6 mm

The rate and depth of respiration while rebreathing is still another factor.

5	respiration	in	25"	Moderately deep	-Carbon	dioxide	=46.4 mm
16	"	"	"	Shallow	"	"	=45.9 mm
5	"	"	"	Very deep	"	"	=45.3 mm
17	"	"	"	Shallow	"	"	=46.4 mm
6	"	"	"	Natural	"	"	=39.0 mm
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with five rather deep inspirations in twenty-five seconds. Series of observations which have compared results obtained by the Plesch method with those obtained by the method of Haldane on the same subject at the same time, show that the Plesch method gives results from one to four milimeters higher than the Haldane. It is claimed by some workers that the advantages of the Plesch-Higgins method are obvious. That since it is difficult to make sick people give an expiration of maximum depth and since anyone can breathe deeply enough to give accurate results with the rebreathing procedure, its use is to be preferred. According to Haldane's results, however, a breath of maximum depth is not required, and the author has found that the dislike of the patient for the rebreathing procedure, requiring as it does the closing of the nostrils, influences unfavorably the obtaining of the Alveolar air by that method.

The possibility of the diffusion of gases through rubber is potentially another source of error where the gas is collected in a thin walled rubber bag. The work of Dewar, however, would indicate that it could not be a disturbing factor where the time between collection and analysis was of brief duration.

The air for the Marriott determination is collected by the Plesch method. The rubber bag, however, as furnished by the dealers is an ordinary football bladder with a very narrow bore inlet tube. Experience has shown that such a narrow inlet prejudices seriously the efficacy of the equilibrium establishment and as a result we have substituted a modification of the Plesch Higgins apparatus just described.

A base for the cap is spun out of aluminum. The edge of this is rolled, serving to hold the cap in place without the use of the rubber band. The three-way brass valve is replaced by a protruding nipple an inch and a quarter in diameter. In this is inserted a rubber stopper thro which a short 3/4 inch glass tube is passed. This tube is connected by thin walled rubber tubing to the mouth-piece, blown from glass tubing of the same bore. The bag is closed by a pinchcock on the rubber tubing. Samples of air are withdrawn thro this same outlet. In cases where patients are not able to cooperate, a mask covering the mouth and nose makes the collection of air possible.

C O N C L U S I O N .

C O N C L U S I O N .

The character of the study imposes certain conditions as criteria for the weighing of the methods to be employed. As has already been stated, a method to be available for the study, must offer, first: simplicity of operation; second: minimum of time consumption; and third; a sufficient degree of accuracy to warrant confidence in the generalizations to be based uoon the measurements.

While none of these factors may be taken for granted and must all be subjected to experimental proof, the third, namely the accuracy of the method, takes the most important place. That just standards may be obtained for comparative purposes, it becomes necessary to adopt a standard method for the determination of Carbon dioxide, and one for the Ammonia in the Urine.

In regard to the first, the wealth of experimental evidence available coupled with a critical analysis of the technique of the method itself, leads to the selection of the Haldane apparatus as one which will give dependable and accurate results.

With the Urinary Ammonia on the other hand the field is by no means so limited, and several methods present themselves, all offering potential accuracy but involving equally possible sources of error. It may be assumed from the conditions of experiment that direct determination in the original solution, where the latter is of so highly complex a nature as is the Urine, offers so

large a number of possible disturbing factors as to render dependence upon it hardly warranted.

Of the indirect methods those involving the use of heat would again seem to be less desirable than those carried on at room temperature, owing to the possibly greater activities of the reagents upon other Urine constituents at the high temperature. By exclusion, one arrives at the Aeration and Precipitation methods and of these the latter, owing to the variability of the essential reagent, is, as has already been mentioned, susceptible to possibly grave error. Of the Aeration methods, the microchemical procedure of Folin-McCallum would seem to give most satisfactory results, both theoretically and practically. The speed of manipulation, and dependability of the same, all point to this method as being best suited to the purposes required.

Standards having been thus determined, the next point is selection of test methods which from their simplicity and rapidity seem to offer the greatest advantage. It is the purpose of the work in process to ascertain the relative accuracy of such methods and so beyond a brief examination of those sources of error, which are obviously incident to the procedure, the first two criteria can be assumed to be the determining factors in the choice.

Considering first the gas analysis, it is evident that a method in which but a single piece of apparatus is needed, offers decided advantage, other things being equal. As was previously stated, the Fridericia tube was designed

specifically for this purpose and naturally offers one of the methods to be investigated. In order to secure samples of gas from this apparatus for exact analysis by the Haldane apparatus, the instrument has been modified by the author in the following way:
The large cock which is closed at the end of expiration thereby sealing the sample within the measuring tube, has been replaced by a three-way cock, the third arm being of capillary dimensions. After the collection of a sample within the tube and the equalization of the temperature of the gas by the usual procedure, mercury can be admitted at the bottom of the apparatus and a sample of the gas passed through the capillary tube into the Haldane apparatus and there subjected to exact analysis. The large bore of the ordinary delivery arm of the apparatus makes this modification necessary as the amount of air contained within it would prove a serious contaminator of the sample and vitiate the analysis made of the mixture.

Of other methods available, the Gravimetric and Thermometric are quite obviously unsuitable. The volumetric method involves standard solutions, burettes, and other accessory pieces of apparatus which makes it not readily portable. Marriott's colorimetric method, on the other hand, offers decided advantage from its portability and simplicity of manipulation and sound theoretical basis. The method of collection of the air sample for this apparatus and naturally for comparison

with the Haldane instrument has already been discussed, the errors incident to the collection considered. The apparatus used by the author for this purpose is, as has already been stated, no more than a modification of the Higgins apparatus and possesses the only advantage over the latter of lightness and slightly simpler manipulation. It is to be regretted that comparative measurements could not be made with the Interferometer, but the instrument has not been available up to the present time, although it is hoped in some of the later series to make use of it. The accuracy calculated, namely .05%, is equal to about 1% of the total amount, and which, as the measurements are reported in terms of millimetres of mercury pressure and vary from 15 to 45, would assume a wholly negligible magnitude.

The work then comprises the determination of the Alveolar Carbon dioxide by means of the Fridericia apparatus, with control determination by the Haldane, on the tension of the gas in arterial blood, and determination on the same patient of the venous tension by means of the Mariott method with the Haldane again being used for control, the various factors of importance as already outlined determined by experiment.

The selection of a method for the determination of Urinary Ammonia is a matter of some difficulty. The direct Nesslerization of the Urine as suggested by Folin and Denis, and Sumner, the direct titration or the so-called "formol" method, and the precipitation method suggested by

Folin and Bell, all present points of desirability. As, however, the two methods suggested by Folin require the use of a colorimeter, an instrument which even in its less expensive forms calls for a considerable outlay of money, this renders them the less desirable from the standpoint of the clinician. The Ronchèse-Malfatti or "formol" titration while, as has been pointed out, ~~is~~ susceptible of certain inherent errors, does not call for an elaborate apparatus and is both rapid and simple. The tenth normal caustic soda which is required is also used for certain other laboratory procedures incident to clinical diagnosis and hence forms a part of even modest laboratory equipment. For these various reasons it was selected as the single comparative method for Urine Ammonia.

The character of the Folin-McCallum aeration is such as to bring it also within the possible range of clinical procedure. This phase of study then consists of the determination of Ammonia content in the 24 hour elimination. As the total amount of the Ammonia is to be regarded as less of an index than the amount in relation to the total nitrogen, this latter factor has also been determined as a matter of routine adopting the thoroughly familiar Kjeldahl method. A sample of the form used for report is here included, and shows the scope of the experimental work as it is being carried out.

In conclusion, the writer wishes to say that in presenting this dissertation, no attempt has been made to include experimental results, as these are at the present

moment in process of compilation and have not reached a point in progress to warrant any conclusions. It is purposed at some later date to present these experimental results and the conclusions drawn from them.

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